Skin barrier structure and function and their relationship to pruritus in end-stage renal disease

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Abstract

Background. The relationship between dry skin and uraemic pruritus remains controversial. In addition, there is a lack of published data describing the structure and function of the stratum corneum (SC) in end-stage renal disease (ESRD). The purpose of the present study was to assess the function and structure of the skin barrier in patients with ESRD and to correlate any abnormalities with uraemic pruritus.

Methods. Thirty-eight subjects participated in the study; 20 with ESRD and 18 healthy controls. Subjects underwent evaluation of SC integrity and permeability barrier recovery, SC surface pH, pruritus and dry skin. The content of glycerol, an important endogenous humectant, was assessed in D-squame tape strips from seven patients with ESRD. Skin biopsies from six of these patients were examined by electron microscopy using ruthenium tetroxide (RuO4)-post-fixation.

Results. Although SC integrity was impaired in ESRD patients ($P=0.001$), there were no significant differences in permeability barrier recovery rates between ESRD subjects and controls. However, there was a high significant negative correlation between SC glycerol content and dry skin in the arms of ESRD subjects ($r=-0.866$, $P=0.01$). Yet, there was no consistent correlation between pruritus and either dry skin, SC integrity, glycerol content or surface pH. Electron microscopy revealed no significant ultrastructural abnormalities, with particular reference to the lipid bi-layer.

Conclusions. SC integrity, but not permeability barrier recovery, is impaired in dialysis patients. Although dry skin in ESRD is associated with reduced SC glycerol levels, the ultra-structure appears to be unaffected.

Keywords: chronic renal disease; electron microscopy; glycerol; permeability recovery; stratum corneum integrity; uraemic pruritus

Introduction

Uraemic pruritus is a most distressing symptom of end-stage renal disease (ESRD), even in patients who are adequately dialysed [1]. Recent epidemiological studies have demonstrated the prevalence of pruritus amongst ESRD patients remains high, ranging between 42% and 75% [1–5]. Despite a plethora of suggested mechanisms, the pathophysiology of uraemic pruritus remains elusive [6–8].

Dry skin (xerosis) is the most frequent cutaneous manifestation of ESRD and has been suggested as a cause of uraemic pruritus [9–13]. Although a positive correlation between the severity of clinical dry skin and pruritus has been shown [11,14], others have failed to find a relationship between either trans-epidermal water loss (TEWL, a measure of the rate of water loss through the skin) or skin hydration and pruritus [15,16]. Current understanding conceptualizes the stratum corneum (SC, the outermost layer of the epidermis) as a ‘brick and mortar’ complex; the corneocytes form the ‘bricks’, while the intercellular lipid bi-layer represents the ‘mortar’ [17]. Abnormalities of the intercellular lipid bi-layer are associated with both dry skin and loss of cutaneous barrier (or skin protective) function [18–20]. In particular, it has recently been shown that the glycerol content of the SC influences cutaneous...
hydration, which independently impacts epidermal barrier function [21,22].

Skin barrier function is often not accurately reflected by basal TEWL measurements in elderly, neonatal and psychologically stressed subjects as well as in dry, scaly (ichthyotic) and uraemic skin [16,23–27]. Acute perturbations of the SC followed by an assessment of the kinetics of barrier recovery may yield a more accurate assessment of epidermal barrier function [23,28]. Surprisingly, there is a paucity of published data about skin barrier function in patients with uraemic pruritus. Moreover, no study has examined whether ultra-structural or biochemical lipid abnormalities within the SC could account for pruritus in ESRD. The purpose of the present study was to assess the function and structure of the skin barrier in patients with ESRD and to correlate any abnormalities with uraemic pruritus.

Subjects and methods

The study protocol was approved by the Wake Forest University Health Sciences Institutional Review Board, NC, USA. Thirty-eight subjects participated in the study of which 22 were female and 16 were male (mean age ± SD: 43.7 ± 11.1 years; range: 22–61 years). Study subjects undergoing haemodialysis were recruited from the High Point Kidney Center in High Point, NC. In addition, 18 healthy volunteers matched for age, ethnicity and gender were enrolled as controls. For demographics and clinical characteristics of the study population see Table 1. All subjects provided written informed consent prior to enrolling in the study.

The diagnosis of ESRD was confirmed by clinical criteria and laboratory results. Subjects with other skin diseases (e.g., atopic dermatitis or psoriasis) were excluded. Subjects were not permitted to apply moisturizers, glycerinated soaps or cleansers to the areas to be examined for 1 week prior to the start of the study. All measurements were performed in a controlled temperature room at 22 ± 0.5 °C and relative humidity of 25%.

All subjects underwent evaluation of SC barrier integrity and permeability barrier recovery, SC surface pH, pruritus and clinical dry skin of the proximal volar forearm. In addition, the glycerol content of the SC from the forearm and anterior shin were measured in seven of the ESRD patients at the time of barrier integrity assessment. Furthermore, six of these ESRD patients underwent a punch biopsy of the skin from the forearm contra-lateral to their vascular access.

### Trans-epidermal water loss (TEWL) measurements

TEWL was measured using two techniques; an open chamber TEWL device—Tewameter TM 300, Courage & Khazaka Electronic, Köln, Germany and a closed chamber TEWL device (VapoMeter, Delfin Technologies Ltd, Kuopio, Finland). Measurements performed using both of these devices are highly correlated [29]. Readings were expressed in g/m²/h. Although both methods were used and there was no statistical difference between the values obtained, measurements from the closed chamber TEWL device were used in the analyses.

### Stratum corneum integrity

SC integrity was measured using tapes from D-Squame (CuDerm Corp., Dallas, TX, USA). These clear adhesive coated discs, with a diameter of 22 mm and a surface area of 38 cm², were used to obtain material for analysis of SC glycerol content and to assess the resistance of the SC to stripping, reflected by the rate of change in TEWL with successive stripping [28,30,31]. Each tape was pressed onto the skin surface with a spring loaded device (D-Squame Pressure Device D500, CuDerm Corp., Dallas, TX, USA) to achieve a standardized pressure of 225 g/m². D-squame tapes were applied to the skin surface of the proximal volar forearm in all subjects and in both the forearm and anterior part of the shin in seven subjects with ESRD. Using this procedure, the SC was removed with repeated D-squame tape stripping until the TEWL reached a value of 20 g/m²/h. The total number of tape stripplings was recorded when the TEWL reached a value of 20 g/m²/h.

### Permeability barrier recovery

We performed 30 tape strippings using D-squame tapes on the skin surface of the proximal volar forearm prior to the haemodialysis session. TEWL was measured with an open chamber (Tewameter TM 300) and closed chamber device (VapoMeter) at five time points: baseline, immediately after tape stripping, 4 h post-tape stripping, day 2 and week 1.

\[
\frac{\text{TEWL immediately after stripping} - \text{TEWL 1 week later}}{\text{TEWL immediately after stripping}} \times 100\%
\]

(TEWL immediately after stripping – TEWL immediately after stripping – baseline TEWL)

### Stratum corneum surface pH

SC surface pH was measured using a flat surface electrode attached to a pH-Meter PH 905 (Courage & Khazaka, Köln, Germany). Measurements were taken at the same 5 time points as in permeability barrier recovery.

### Clinical assessment of itch

The severity of itch was assessed at screening by a 10 cm visual analogue scale (VAS) at four different states: current, worst itch, best state and itch intensity after a mosquito bite (reference state). This method has been described previously [32].

<table>
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<tr>
<th>Table 1. Demographics of the study population</th>
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<td><strong>Dialysis patients</strong></td>
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<td>Dry skin, n (%)</td>
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<td>Pruritus</td>
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<td><strong>Healthy controls</strong></td>
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<td>11 (61%)</td>
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<td>Dry skin, n (%)</td>
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<td>5 (28%)</td>
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<td>Pruritus</td>
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Clinical assessment of dry skin (xerosis)

Clinical dry skin was assessed at the time of screening with a 5 point Likert scale ranging from 0 = no dryness to 4 = severe dryness.

Biopsies and ultrastructural evaluation

Six subjects with ESRD underwent 3 mm cutaneous punch biopsies of the forearm contra-lateral to their vascular access site. Three of these patients reported pruritus, while three did not and dry skin ranged from mild to severe.

After aldehyde pre-fixation of 3 mm punch biopsies, samples were post-fixed with ruthenium tetroxide (RuO₄), followed by dehydration and embedding by lipid retaining epoxy resin. Ultrathin sections were examined and photographed in a Zeiss 10A electron microscope operated at 60 kV.

Stratum corneum glycerol content analysis

To determine glycerol content of the SC, D-squame tapes from seven subjects with ESRD were analysed. SC glycerol content was evaluated using sequential 22 mm D-Squame tape stripplings (n = 30) pooled and soaked in 400μl of 1% Triton X-100 (Sigma, St Louis, MO, USA) in water, as previously reported [21]. Glycerol content was expressed as nanomoles per 30 D-Squames.

Statistical analysis

All statistical analyses were performed using SPSS 14.0 with statistical significance set at P < 0.05. Between group comparisons (ESRD vs healthy controls) in skin barrier integrity, skin barrier recovery and skin surface pH were assessed using two sample t-tests if normality and homogeneity assumptions were satisfied, otherwise the non-parametric Mann-Whitney U test was performed. The rate of skin barrier recovery within groups was assessed using repeated measurements. Paired t-test or its non-parametric equivalent—Wilcoxon’s signed rank test compared the glycerol content between the arms and legs, as well as the within-group analysis on the skin surface pH for each time point and at baseline. Spearman’s correlation analysis was performed for the number of tape stripplings with the presence of uraemic itch, skin pH with presence of pruritus, the glycerol content with itch and dry skin severity, and between skin dryness and barrier integrity. Chi-square tests were performed to determine the association between skin dryness in ESRD patients, with odds ratios presented.

Results

Stratum corneum integrity

SC integrity was impaired in ESRD patients compared with healthy volunteers (mean number of tape stripplings to achieve 20 g/m²/h of TEWL ± SD: 38.1 ± 25.6 vs 61.7 ± 25.0, P = 0.001, respectively). We did not find any correlation between the number of tape stripplings and the presence of uraemic itch (Figure 1).

Permeability homeostasis

As expected, TEWL levels were higher in ESRD patients after the 30 tape stripplings and 4h post-stripping (P = <0.05), indicative again of abnormal SC integrity (Figure 1). However, there was no difference in permeability barrier recovery rate between ESRD patients and healthy controls (Figure 2).

Stratum corneum surface pH

At baseline, SC surface pH was slightly higher in ESRD subjects compared with controls; however, this difference was not statistically significant. There was no correlation between SC pH and the presence of pruritus in ESRD subjects.

Glycerol content of the stratum corneum and clinical skin dryness

There was a significant negative correlation between SC glycerol content and dry skin in the arm (r = −0.866, P = 0.01), although we did not detect...
this relationship in the SC of the leg. There was no correlation between SC glycerol content and pruritus in either the leg or the arm.

Skin dryness was significantly more common in ESRD patients (75%, \( n = 15 \)) when compared to healthy volunteers (28%, \( n = 5 \)) (OR = 7.8, 95% CI 1.8–33.1, \( P = 0.004 \)). There was no correlation between dry skin and pruritus in ESRD patients. Furthermore, we did not find any association with the degree of skin dryness and barrier integrity.

**Ultrastructural analysis of stratum corneum**

Ultrastructural analysis revealed no abnormalities in the SC–stratum granulosum interfaces or the cornified cell envelopes. In addition, the lamellar body number and secretion were unaffected.

**Discussion**

Abnormal skin barrier integrity suggests a loss of cohesion between corneocytes, thus one would expect to see changes in the structural organization of lipids in the SC. The current study demonstrates abnormal skin barrier integrity in patients with ESRD. However, no disturbances in the ultrastructure of the SC, particularly the intercellular lipid bi-layer, were detected. We detected a negative correlation between the glycerol content of the SC and dry skin in the arm, although we were unable to replicate this result in the SC of the leg.

Recent data suggest that endogenous glycerol of both sebaceous gland and non-sebaceous gland origin (possibly from blood) positively influences SC hydration in humans [21]. AQP3 (aquaporin-3), an integral membrane channel in the basal layer of epidermal keratinocytes, facilitates glycerol transport from the circulation into the epidermis [33]. Mice deficient in AQP3 demonstrate abnormal hydration and reduced glycerol content of the SC [34]. Interestingly, a significant reduction in the renal expression of AQP3 has been shown in rats with both acute and chronic renal failure [35,36]. This observation may provide a plausible explanation linking ESRD with dry skin, a clinical finding which is more frequent in patients on dialysis. Additionally, serum lipid abnormalities are frequent among ESRD patients [37] and may influence lipids in the SC, where they play a central role in dry skin and epidermal barrier function. This possible association would be an interesting area to explore in future studies.

Skin barrier recovery is enhanced after topical acetone treatment (to mimic dry skin conditions) in humans [38]. Previous studies examining basal TEWL in uremic subjects did not reveal significant differences from healthy controls [16]; however, basal TEWL measurements often do not accurately reflect skin barrier status [23,28]. In the current study, SC integrity and permeability barrier recovery using tape stripping were evaluated. Although SC integrity was abnormal, we found permeability barrier recovery rates to be normal in ESRD patients.

In addition, we found that SC surface pH tended to be higher among ESRD subjects compared with healthy controls, although this finding did not achieve statistical significance. Elevated skin surface pH has been demonstrated previously in ESRD [15], however, the exact mechanisms are unknown. A defect in the non-energy-dependent sodium–proton exchanger (NHE 1) in keratinocytes could be causative [39]; however, this requires further investigation. Elevated SC pH induces activation of serine proteases which in turn leads to abnormal barrier function and SC integrity [40]. This effect is believed to be mediated by degradation of lipid processing enzymes and corneodesmosome proteins. This finding could explain the high prevalence of dry skin among ESRD patients.

Pruritus remains a common and disabling symptom of ESRD. The present study failed to detect a correlation between dry skin and pruritus in ESRD patients, a finding that contrasts with other reports [11,14] but is in agreement with bioengineering studies that assessed TEWL and skin hydration [15,16]. We also found no correlation between skin barrier integrity and pruritus, despite the knowledge that damage to the SC elicits a scratching response [41]. Furthermore, there was no association between the glycerol content of the SC in ESRD patients and pruritus. Serine proteases, via their activation of protease-activated receptor 2, have been shown to be involved in itch induction [42]. Given that the activity of these enzymes is enhanced with higher SC pH (as above), one would expect an increase in pruritus with an alkaline skin surface. The present study did not demonstrate a significant correlation between skin pH and pruritus. In the future, it will be important to examine serine protease levels in patients with ESRD.

Skin barrier integrity, but not permeability barrier recovery, is impaired in patients with ESRD. However, this observation was not accompanied by detectable abnormalities in the ultra-structure of the SC, particularly in the intercellular lipid bi-layer.

**Conflict of interest statement.** None declared.

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